Phytochemical and Molecular Characterization of *Tylophora indica* (Burm.F) Merrill.

A Synopsis of the proposed work for
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INTRODUCTION

People are exploring the plant species in search of new drugs, which has resulted in exploitation of large number of medicinal plants with curative properties to treat various ailments. The importance of medicinal plants has become more potent now in developing countries.

In India, it is estimated that 80% of the population depending on plants to therapy themselves, of those about 60% population use medicinal plants habitually to battle certain ailments and almost 40% human use such plants in pharmaceutical industries (Hiren, 2013). The World Health Organization (WHO) has outlined herbal medicine as culminated labelled medicinal products (Romila, 2010; Dash *et al*., 2017).

Plants have the ability to produce diverse variety of phytochemical compounds which can be used to perform different biological functions. Many of these phytochemicals have beneficial effects on long-term health when consumed by the humans and can effectively use to treat human diseases.

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. They are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs (Ncube *et al.,* 2008; Chaudhuri, 2009). Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011). The pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants (Brindha, 2012).

There is an increasing demand for plant based medicines to control many of the diseases. Some medicinal plants, having lot of medicinal value, but their phytopotential value are not explored completely till date. Among them one of the plant is *Tylophora indica*, an

important medicinal plant of India. Dried leaves and roots are regarded as one of the best indigenous substitutes for the Ipecacuanha (*Cephalis ipecacuanha*) used for the treatment of respiratory troubles (Umamaheswari, 2017).

Tylophora indica (Burm.F) Merrill is a useful medicinal plant, previously called as *Tylophora asthamatica* (Rani *et al*., 2012) commonly known as Antamul, Indian Ipecac Damnibel, Dam bel and belongs to family Asclepiadaceae. It is indigenous to India found in the sub-Himalayan tract extending from Uttar Pradesh to Meghalaya (Kaur and Singh 2012). The plant is found growing normally in Bengal, Assam, Orissa, South India, North east and Central India (Joshi, 2000). It is a perennial, branching climber or shrub that grows up to 1.5 meters, leaves are obviate-oblong to elliptic-oblong, 3-10cm long and 1.5-7cm wide. Roots Long fleshy with longitudinally fissured light brown, corky bark. (Kirtikar and Basu, 2001; Gupta, 2003; Vivean *et al*., 2014).

The leaves and roots of the plant contain 0.2-0.46% therapeutically important alkaloids *viz*., tylophorine ($C_{24}H_{27}O_4N$), tylophorinine ($C_{23}H_{25}O_4N$) and tylophorinidine ($C_{22}H_{22}O_4N$) (Kaur and Singh, 2012; Kaur *et al*., 2016). Some other alkaloids are tyloindicine I (potential anticancer alkaloids), septicine, isotylocrebrine, tylophoricine, sterols, flavanoids, wax, resins and tannins (Rani *et al*., 2012). Recently some rare alkaloids namely tyloindicines A, B, C, D, E, F, G, H, I, and J, desmethyltylophorine, desmethyl tylophorinine, isotylocrebrine, anhydroustylophorinine, anhydrousdehydrotylophorinine, a- fagarine, skimmianine, 14 -hydroxyisotylocrebrine, 4,6- desmethylisodroxy-o- Methyltylophorinindine have been reported. The non-alkaloidal compounds isolated from *T. indica* are kaempferol, quercetin, octaosanyl octacosanoate, sigmasterol, â-sitosetrol, tyloindane, cetylalcohol, coutchone, pigments, glucose, calcium salts, potassium chloride, quercetin and kaempferol. Steam distillation of an alcoholic extract of the air dried root powder gave p- methoxy salicyaldehyde and a small amount of oily matter. (Ali, 2008; Vivean *et al*., 2014).The leaves

and roots of *Tylophora* are used as a source of bioactive material. It is reported to have laxative, expectorant, diaphoretic, purgative, stimulant, emetic and cathartic properties.

Traditionally it is used as folk remedy in treatment of bronchial asthma, bronchitis, rheumatism, allergies, inflammation (Faisal and Anis, 2005; Kaur *et al*., 2011), dysentery, dermatitis (Singh *et al*., 2009), whooping cough, diarrhea, snake bite, muscle relaxant. This plant also shows some pharmacological activity *viz*., antimicrobial activity, diuretic, hepatoprotective, immunomodulatory (Kaur and Singh, 2012), anticonvulsant, antifungal, anti diabetic, anti tumor, antioxidant, antihistaminic, antipsoriasis etc. (Vivean *et al*., 2014).

It has reputation as alterative and as a blood purifier often used in rheumatism. Dried leaves are emetic diaphoretic and expectorant. The leaves and roots are also used in hydrophobia. Leaves are employed to destroy worms and the leaf extract act as anti-tumor. The roots are suggested to be a good natural preservative of food. *Tylophora* species are now in great demand in worldwide because of their proven efficacy against asthma. An ayurvedic nutriceuticals, Sabina corporation, a U.S. company produced standardized extract of *Tylophora indica* having composition of 0.1% alkaloid used for respiratory disorders. Ayush Herbs Inc. company marketed *Tylophora* plus capsules is an ayurvedic herbal formulation designed to support the lungs. The combination of *Tylophora indica*, *Piper longum*, *Zingiber officinale* and *Emblica officinalis* have been shown to support the body's immune function (Rani *et al*., 2012).

The commercial cultivation of this important medicinal plant species is uncommon and the destruction of plant caused by harvesting the roots as a source of drug has threatened the very existence of this plant (Faisal and Anis, 2003, Thomas and Philip, 2005; Kaur *et al*., 2014).

Most of the works reported in literature are associated with micropropogation and little works done on phytochemical analysis of plant. There is no report on its metagenomics and transcriptomics.

The aim of this study will be to study the important active ingredients of plants and identify the potential transcripts and provide a framework of identified transcripts involved in alkaloids production through transcriptome assembly. The identification of transcripts study will establish a foundation for future studies related to carrying out the metabolic engineering for increasing the tylophorine biosynthesis and its regulation for human health benefits. Metagenomics will explore the microbial community associated with the soil rhizosphere of *Tylophora indica.*

OBJECTIVES

All parts of *Tylophora indica* are extensively collected for medicinal purposes. This is why plant has become threatened in the most part of India. In view of this the present work has been taken in the following steps:

1. Phytochemical characterization of various plant part of *T. indica*.
2. The study on bacterial diversity associated with the rhizosphere of *T. indica*.
3. *De Novo* assembly and Transcript profiling of *T. indica*.

REVIEW OF LITERATURE

A thorough, review of literature has been conducted for making a sound and concrete basis to frame present researck work.

Dash *et al*., (2017) stated that phytochemical analysis of methanolic leaf extracts of *Azadirachta indica* has shown the presence of biological compounds like, Alkaloids, Flavonoids, Saponins, etc which are then compared to aqueous leaf extracts of the plant. Biochemical analysis includes the estimation of chlorophyll content, carbohydrate content and proline content.

Gayathri and Shilpa (2017) revealed the presence of various biochemical compounds such as chlorophyll, protein and carbohydrate from *Annona squamosa* L. and *Garcinia gummi-gutta* (L.) Roxb. It was found that the chlorophyll 'a', chlorophyll 'b', total chlorophyll and protein content was significantly higher in *Annona squamosa* L. The carbohydrate content was found to be higher in *Garcinia gummi-gutta* (L.) Roxb.

Rao and Kaladhar (2014) tested tuber extracts of *Globba bulbifera* for phytochemical and biochemical studies. Ethanolic extract shown good number of compounds compared to methanolic, ethyl acetate, aqueous and chloroform extracts. Phytochemical analysis of ethanolic tuber extract of *Globba bulbifera* has shown the biological compounds like carbohydrates, cholesterol, Amino acids, Steroids, Alkaloids, Flavonoids, Cardiac glycosides, Saponins, Tannins, Terpenoids, Phlobatinins, Fattyacids, Coumarins and Phenols.

Wang *et al*., (2018) compared the rhizosphere microbial community of four *Ferula* species *F. syreitschikowii* K.-Pol., *F. gracilis* (Ledeb.) Ledeb., *F. ferulaeoides* (Steud.) Korov., and *F. lehmannii* Boiss. The 16S rRNA sequences of rhizosphere bacteria were obtained

with an Illumina paired-end sequence platform. Analysis was conducted to determine the richness and diversity of the rhizosphere bacterial communities.

Mukherjee *et al*., (2017) explained that *Bacillus sp.* which was isolated from the rhizospheric soil of *Withania somnifera*, possess multiple plant growth promoting activities. *Bacillus sp.* was screened for their plant growth promoting activities like phosphate solubilisation, production of Indole acetic acid (IAA), ammonia, hydrogen cyanide (HCN), catalase, cellulase, biofilm, siderophore and organic acid. Different *Bacillus* species like *Bacillus sonorensis, Bacillus subtilis, Bacillus pumilus,* and *Bacillus licheniformis* were identified by biochemical characterization. Their PGPR activity concluded that *Bacillus subtilis* showed maximum amount of PGPR traits, *Bacillus sonorensis* showed optimum and *Bacillus pumilus* showed minimum amount of PGPR traits, therefore, *Bacillus subtilis* is the best plant growth promoting rhizobacteria (PGPR) with maximum PGPR traits.

According to Badry, (2016) the microbes play a role in plant growth, nutrient availability, disease resistance, yield and quality of medicinal compounds. Three rhizospheric medicinal plants were collected from Saint Katherine Mountain, Sinai, Egypt. Total DNA was extracted from sand samples and characterized its bacterial communities using the terminal restriction fragment length polymorphism method and degrading gradient Gel electrophoresis. This work revealed that water changed bacterial community in all samples compared with dry sand due to water may induced shifts in bacterial community. Bacterial community clone library of *Capparis spinosa* rhizosphere were studied and phylogenetic tree of bacterial community were evaluated.

Ernawati *et al*., (2016) aimed to assess the community structure of actinobacteria in rhizosphere and endophyte of a medicinal plant, *Centella asiatica,* based on a metagenomic approach using Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-

DGGE) of 16S rRNA gene. The results of separation in DGGE gel showed 16 major bands from rhizosphere and plant tissue. The bands distribution pattern showed that the community of actinobacteria in the plant tissue was slightly more diverse than rhizosphere.

Akinsanya *et al*., (2015) stated that Next generation sequencing (NGS) enables rapid analysis of the composition and diversity of microbial communities in several habitats. The use of high throughput techniques of NGS to the metagenomics study of endophytic bacteria in *Aloe vera* plant, by assessing its PCR amplicon of 16S rDNA sequences (V3–V4 regions) with the Illumina metagenomics technique used to generate a total of 5,199,102 reads from the samples.

Velázquez *et al*., (2012) analyzed the bacterial diversity associated with the rhizosphere of wheat plants by PCR amplification, construction of a library and sequencing of 16S rDNA genes. Thirty OTUs were detected, including the Classes Alfaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobateria, Actinobacteria, Bacilli, Clostridia and Uncultivable bacteria. Within the Gammaproteobacteria class, the genera Pseudomonas, Stenotrophomonas and Bacillus were the most abundant.

Burnii *et al*., (2011) studied the AMF relationship with chemical characteristics of rhizospheric soils of *Mentha arvensis* and M *longifolia,* growing in some cultivated and natural sites of KPK (Khyber Pakhtun Khawa) respectively. Seven AMF species with *M. arvensis* and 11 with *M. longifolia* were recorded. The dominant Genus was *Glomus* (11 species) followed by *Acaulospora and Gigaspora*.

Lidija *et al*., (2010) studied the composition and size of bacterial communities of *Ramonda serbica* and *Ramonda nathaliae* rhizospheric soil samples and analyzed using a metagenomic approach. Using universal bacteria – specific primers 16S rDNA genes were amplified directly from metagenomic DNAs and two libraries were constructed. The Restriction

Fragment Length Polymorphism (RLFP) method was used in library screening. Amongst 192 clones, 35 unique operational taxonomic units (OTUs) were determined from the rhizosphere of *R. nathaliae*, and 13 OTUs out of 80 clones in total from the library of *R. serbica*.

Song *et al*., (2017) studied two *Salvia miltiorrhiza* genotypes (BH18 and ZH23) with different phenolic acid concentrations were used for de novo RNA sequencing (RNA-seq). A total of 170,787 transcripts and 56,216 unigenes were obtained.

Rai *et al*., (2016) described Illumina based RNA sequencing analysis to characterize the transcriptome and expression profiles of genes expressed in the five tissues of *Panax japonicus*, and its comparison with other *Panax* species. RNA sequencing and de novo transcriptome assembly for *P. japonicus* resulted in a total of 135,235 unigenes with 78,794 (58.24%) unigenes being annotated using NCBI-nr database.

Zhang *et al*., (2015) presented the sequence and transcript abundance data for the root and leaf transcriptome of *G. rigescens*, obtained using the Illumina Hiseq2000. Over fifty million clean reads were obtained from leaf and root libraries. This yields 76,717 unigenes with an average length of 753 bp. Among these, 33,855 unigenes were identified as putative homologs of annotated sequences in public protein and nucleotide databases. Digital abundance analysis identified 3306 unigenes differentially enriched between leaf and root.

Misra (2014) stated that plant genomes and transcriptomes, provides an opportunity to identify genes involved in secondary metabolic pathways in many plant species of agronomical interest. Most genome sequencing attempts have focused on model plants i.e., dicot model *Arabidopsis thaliana*, monocot model rice (*Oryza sativa*), unicellular viridiplantae *Chlamydomonas*, woody plant model *Populus trichocarpa* and mostly other commercially beneficial plants such as Coffee, Soybean, Castor bean, Strawberry, Maize, Sorghum, Papaya, Grape, Cotton, Ginseng, Apple, Rubber, Cucumber and others. Thus

medicinal plant genomics has been a less-focused field of investigation, apart from the sequenced genomes of neem and hemp.

Meredith *et al*., (2011) conducted a whole transcriptome analysis on root tissues of *Arabidopsis thaliana* and categorized genes with significant changes in expression. Genes from four categories of interest based on significant changes in expression were identified as ATP/GST transporter, auxin/auxin related, secondary metabolite and pathogen response genes.

METHODOLOGY

1. Phytochemical characterization of various plant part of *Tylophora indica*.

For Phytochemical characterization different plant parts *viz*., leaves and roots of *T. indica* will be used for extraction following the method (Dash *et al*., 2017).

a. Plant sample will be collected, washed with distilled water and air dried at room temperature.
b. The dried plant material will pulverized and stored in air-tight containers at 4˚C.
c. Dried plant material will be used for extract preparation by using solvents.
d. Then the prepared extract used to be taken for phytochemical evaluation such as alkaloids i.e. Tylophorine by following the method (Gupta *et al*., 2012).

2. The study on bacterial diversity associated with the rhizosphere of *Tylophora indica*.

For analysis of bacterial diversity method of (Velázquez *et al*., 2012) will be followed.

a. Total DNA extraction of rhizospheric soil sample.
b. Polymerase chain reaction amplification of the bacterial 16S rRNA genes and contruction of the library.
c. Sequencing and analysis of the 16S rDNA genes.
d. Phylogenetic and statistical analysis.

3. *De Novo* assembly and transcript profiling of *Tylophora indica*.

For the transcriptomics analysis isolation of mRNA from various parts of the *Tylophora indica,* library preparation and sequencing will be characterized by following the method (Han *et al*., 2016).

a. Isolation of Total RNA by using commercially available RNA preparation kit.
b. RNA quality assessment and purification will be quantified by using spectrophotometer.
c. Transcriptome profiling, cDNA will be synthesized from mRNA for sequencing.
d. Validation and quantification of the cDNA library.
e. *De novo* sequencing- The next generation sequencing would be performed. High quality data of plant sample will be assembled by using RNA sequence assembler on optimized parameters.
f. Data analysis by using bioinformatics tools.

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